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Association of Human Papillomavirus with Cutaneous Squamous Cell Carcinoma and Basal Cell Carcinoma: a systematic review and meta-analysis

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NOME

António Álvaro Pereira de Pinho

NÚMERO DE ESTUDANTE	E-MAIL
20120201	mimed12248@med.up.pt

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TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

Association of Human Papillomavirus with Cutaneous Squamous Cell Carcinoma and Basal Cell Carcinoma: a systematic review and meta-analysis

ORIENTADOR

Prof. Dra. Carmen Maria Lisboa da Silva

COORIENTADOR (se aplicável)

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É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☒
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“Não sei o que nos espera mas sei o que me preocupa: é que a medicina, empolgada pela ciência, seduzida pela tecnologia e atordoada pela burocracia, apague a sua face humana e ignore a individualidade única de cada pessoa que sofre, pois embora se inventem cada vez mais modos de tratar, não se descobriu ainda a forma de aliviar o sofrimento sem empatia ou compaixão.”

João Lobo Antunes
in "A Nova Medicina"

Association of Human Papillomavirus with Cutaneous Squamous Cell Carcinoma and Basal Cell Carcinoma: a systematic review and meta-analysis

Running Head: Association of HPV with Cutaneous Squamous Cell Carcinoma and Basal Cell Carcinoma.

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Authors: A.A. De-Pinho[1], A.L.C. Ramalho[2,3], J.V. Santos[2,4,5], A. Freitas[2,4], C. Lisboa[1,4,6]

[1] Division of Microbiology, Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal

[2]MEDCIDS (Department of Community Medicine, Information and Health Decision Sciences), Faculty of Medicine, University of Porto, Portugal.

[3]FGV (Getúlio Vargas Foundation), Rio de Janeiro, Brazil

[4]CINTESIS (Center for Health Technology and Services Research), Porto, Portugal

[5]Public Health Unit, Aces Grande Porto VIII, Espinho/Gaia, Portugal

[6] Dermatovenereology Department, Centro Hospitalar S. João, EPE, Porto, Portugal

Corresponding author: António Álvaro Pereira de Pinho - mimed12248@med.up.pt; Alameda Professor Hernâni Monteiro 4200-319, Porto, Portugal; Fax: +351220426870.

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Abstract

Background The association between human papillomavirus (HPV) and Non-Melanoma Skin Cancer (NMSC) remains undefined, with studies showing controversial results.

Objective We aim to determine if there is a significant association of HPV with Cutaneous Squamous Cell Carcinoma (cuSCC) and Basal Cell Carcinoma (BCC) and whether the prevalence of HPV is higher in those tumors from immunosuppressed patients when compared with tumors from immunocompetent patients.

Methods We conducted a systematic review and meta-analysis of observational studies. We searched four electronic databases up to March 2017, reviewed references of relevant articles and hand searched conference proceedings. The following search terms were used: skin cancer, squamous cell skin carcinoma, basal cell skin carcinoma and human papillomavirus. Only studies including biopsy samples evaluated by a pathologist, HPV detection by polymerase chain reaction (PCR) and a minimum of 10 cases and 10 controls were considered. Pooled effect size and 95% confidence intervals were calculated using random effects meta-analysis and the inverse variance method.

Results Of 2740 articles identified, 23 met the eligibility criteria. cuSCC were more likely to carry HPV than normal-appearing skin - pooled odds ratio (OR) 3.29 (95% CI 1.67-6.46). The overall association between HPV and BCC was also significant - pooled OR 2.56 (95% CI 1.48-5.14). An increase in HPV prevalence was found in cuSCC tumors from immunosuppressed patients when compared with immunocompetent patients - pooled OR 3.07 (95% CI 2.08-4.52). The same prevalence was not significant in BCC - pooled OR 2.38 (95% CI 0.95-5.96).

Conclusion These results support those studies that reported an association between HPV and NMSC, particularly in cuSCC and in tumors from immunosuppressed patients. Such evidence may have future research and clinical implications.

Keywords human papillomavirus; squamous skin cell carcinoma; basal skin cell carcinoma; meta-analysis; immunosuppression; systematic review.

Introduction

Non-Melanoma Skin Cancer (NMSC) comprises Basal Cell Carcinoma (BCC) and Cutaneous Squamous Cell Carcinoma (cuSCC) as the major representatives.[1] BCC is the most common skin cancer[2] and is the most prevalent cancer in many countries worldwide.[3] On the other hand, cuSCC is the second most frequent form of skin cancer.[4] Reports from the World Health Organization estimate an occurrence of 2 to 3 million cases of NMSC annually worldwide.[5] The annual cost of treating skin cancers is estimated at $8.1 billion, $4.8 billion for NMSC in the United States.[6] NMSC is also a significant economic burden in European health expenditure, with €5.2 million annually costs for inpatient care attributable to these cancers in Sweden[7] and €105 to €130 million estimated annually costs of hospitalization in Germany.[8]

Ultraviolet Radiation (UVR) exposure is the main recognized risk factor for NMSC. About 90 percent of NMSC are associated with exposure to UVR from the sun[9], but other risk factors have been studied as fair skin, older age and immunosuppression.[10] For instance, organ transplanted patients are approximately 100 times more likely to develop cuSCC than general population.[11] Considering the increased prevalence and the clinical behavior of NMSC and the high incidence of virally induced neoplasia such as Kaposi Sarcoma (Human Herpesvirus 8) in this cluster of patients, a viral etiology has also been hypothesized[12, 13] associated with NMSC.

More than 170 types of small capsid-enclosed double-stranded DNA human papillomaviruses (HPV) have been described.[14]Taxonomy recognizes five main HPV genera: alfa-, beta-, gamma-, mu- and nu- viruses.[15] Their oncogenic potential has been studied both *in vitro* and *in vivo* models demonstrating a well-established role of alfa-HPV in cervical carcinoma in an important portion of other anogenital, head and neck and oropharyngeal malignancies.[16]

In the last decades, the biology and contribution of HPV to NMSC development in previously healthy skin has been studied but controversial results were found and different methodological approaches were used.

Public health interventions have been launched to minimize the burden of HPV-associated malignancies, including worldwide vaccination programs against the most oncogenic viruses.[16] A better understanding of the relation between HPV and NMSC

would help to develop more effective preventive measures (such as vaccination) decreasing, therefore, the number of newly diagnosed cases and consequently the financial burden.

The main aim of the present study was to perform a systematic review and meta-analysis to determine the association of HPV with cuSCC and BCC. We also sought to ascertain whether the prevalence of HPV is higher in those tumors from immunosuppressed patients when compared with NMSC from immunocompetent patients.

Material and methods

A systematic review and meta-analysis of observational studies focusing on the association between NMSC and the presence of human papillomaviruses in skin was performed. The methodology included definition of selection criteria and search strategy, quality assessment, data abstraction, bias risk assessment and statistical analysis. PRISMA statement was followed.[17]

Selection criteria

The study eligibility criteria were determined before data collection in order to properly identify high quality studies appropriate for the analysis.

The following eligibility criteria were defined. Patient population: immunocompetent or immunosuppressed patients presenting healthy normal skin (controls) or non-anogenital NMSC lesions including Keratoacanthoma (KA), *in situ* cuSCC, invasive cuSCC or BCC (cases) reported by a pathologist, without other cutaneous comorbid conditions (Epidermolysis Verruciformis or other genodermatosis). Study design: observational studies with a minimum of 10 cases and 10 controls or a minimum of 20 patients. Exposure: HPV presence in skin detected by polymerase chain reaction-based (PCR) methods in biopsy samples. Outcome: NMSC occurrence in both immunocompetent and immunosuppressed subpopulations (Table 1). *INSERT T1*

Search strategy

Our primary method to identify potentially eligible studies was an electronic biomedical literature search in the MEDLINE database, considering articles published until to March 10, 2017, written in English, using the following search keywords (skin cancer or squamous cell skin carcinoma or basal cell skin carcinoma) and human papillomavirus and MeSH terms: “skin neoplasms”, “carcinoma, squamous cell”, “carcinoma, basal cell” and “papillomaviridae”. Literature search was also performed, using the previous search keywords, in other three major databases: the Cochrane Library; the SciVerse Scopus; and the Web of Science.

We also reviewed the references list of all pertinent articles to recognize potentially appropriate studies. Abstracts from relevant conferences and scientific forums were also searched.

Duplicated articles were excluded and all the articles were uploaded to Covidence systematic review manager for quality assessment and further selection.

Quality assessment and data abstraction

During the first phase of selection, each article was independently reviewed by two of the authors, based on its title and abstract to screen for relevance.

In the second phase, articles were independently full-text scrutinized by two reviewers, De-Pinho A.A. and Lisboa C. Selection criteria were applied, exclusions were decided and disagreements settled by consensus.

A standard data abstraction form was created by De-Pinho A.A. and Santos J.V. Each article was checked independently and all relevant data was extracted independently and in duplicate for each article by De-Pinho A.A. and Ramalho A.L.C. Any discrepancies in the duplicates were solved by the authors by discussing and achieving consensus.

General data extracted from each study included authors' names, year of publication, general study design, studied malignancies, tumour body locations, HPV subtypes that were searched, total numbers or HPV positive/HPV negative samples of cuSCC, BCC, normal skin, cuSCC or BCC in immunosuppressed or immunocompetent patients, depending on the hypothesis tested as well as other relevant information concerning methodological specificities to facilitate the assessment of bias risk. Extra data relative to age, sex, race, skin type, sun exposure, immunosuppression and PCR methodology details were also collected when available (Table 2). *INSERT T2*

Following the Newcastle-Ottawa Scale (NOS), the risk of bias in all reviewed studies was assessed regardless of the anticipated variability in outcomes or the validity of the included studies. Two reviewers performed the NOS assessment individually.

Evaluations were performed according to manual coding for observational studies, covering the domains: selection, comparability and exposure. Dispute resolutions between the two reviewers were conducted by consensus method.

Statistical methods

For the assessment of the effect of HPV in the development of cuSCC or BCC, as well in the immunosuppression of both, we used the generic inverse variance method for both fixed and random effects estimation.

Generic inverse variance was used as data type for measuring the HPV effect in the outcomes as it is the optimal measure to describe retrospective exposure effects. Odds ratio (ORs) with the corresponding 95% confidence intervals (CI) were abstracted from each article. We identified the ORs reflecting the greatest degree of adjustment for possible confounding factors. When adjusted values were absent, we collected crude values. As a last resort, if ORs were not mentioned in the selected study, we calculated them based on the number of cases and controls described in the study. Random-effects method was used to pool the ORs and 95% CIs.

Heterogeneity of exposure effect was assessed by graphical inspection of forest plots and formally using the Q statistic (at a p value <= 0.1) and I^2 statistic for studying studies' consistency.

Potential publication bias was assessed by visual analysis of funnel plots and analysis using Begg test.

Data processing and statistical analysis were performed using Review Manager (RevMan) software version 5.3.

Results

Search and study selection

The literature search protocol described above yielded a total of 2740 studies. During this process, two previous meta-analysis on the subject were identified – Wang et al[18] and Chahoud et al[19] and articles included in those studies were selected for our screening. No abstracts from relevant conferences and scientific forums were found.

After review of the titles and abstracts, a total of 2577 were excluded for non-compliance with the eligibility criteria. De-Pinho A.A. and Lisboa C. reviewed the full text of the remaining 163 articles. Selection criteria were applied, exclusions were decided and disagreements settled by consensus. After the final screening, 23 studies were selected (Fig. 1). *INSERT F1*

Study characteristics

All the 23 included studies in our meta-analysis were case-controls (no cohort studies were identified). Depending on the outcome, a different number of studies was considered for statistical analysis (Fig. 1; Table 2). Some studies were included in more than one hypothesis analysis as they addressed more than one outcome.

Publication years ranged from 1996 to 2014. All studies included both male and female patients ranging all ages and resulted in a total of 558 cases and 907 controls for hypothesis A, 370 cases and 667 controls for hypothesis B, 444 cases and 286 controls for hypothesis C and 72 cases and 42 controls for hypothesis D.

After data collection, we recognized that, depending on the study, “cuSCC samples” included invasive cuSCC and/or in situ cuSCC and/or Keratoacanthoma. “Normal skin” was a reference to normal skin tissue from patients with cuSCC/BCC or normal skin samples from paired controls without tumours, depending on the studies, but always reported by a pathologist. Benign lesions were not included.

Some studies included in this meta-analysis evaluated several body locations, namely anogenital lesions.[20] Since those cases did not meet our eligibility criteria, they were excluded. This may explain a discrepancy between the values present in our meta-

analysis and the ones described in the original study. Samples that did not described the specific body location of the lesion or mentioned it only as “unknown” were included.

All the selected studies used PCR-based methods to identify the HPV presence in skin biopsies, but the genera/subtype detected was different among the case-controls, varying from broad spectrum, including alpha, beta and even gamma genera to only a specific HPV subtype.[21]

Only two of the selected studies presented adjusted OR: Forslund et al[22] – adjustment for age, sex, skin type, self-reported previous sunburns, eye colour and sun exposure; and Iftner et al[23] – adjustment for age, sex and sun exposure.

Additional information and abstracted data from the included studies are presented in Table 2.

Methodological quality of included studies

Quality assessment was performed using the NOS, covering the following domains: selection, comparability and exposure. Scores ranged from 5 to 9 (9 being the highest possible score), with a mean of 8.3 and median of 9 (Table 3).

Despite the presence of some possible bias in five studies[23,24,25,26,27] which scored 7 or less, they were relevant enough to be included. *INSERT T3*

Publication bias

Considering the existence of approximately symmetrical funnel plots among the studied hypothesis B, C and D, selected studies from these hypotheses seemed to show no evidence of publication bias. On the other hand, the funnel plot regarding hypothesis A showed some grade of asymmetry. For a better understanding, Begg test was applied to the four funnel plots and detected no evidence of publication bias among the four studied hypothesis (Fig. 2). *INSERT F2*

Meta-analysis

Hypothesis A - HPV association with cuSCC *versus* normal skin

A total of 11 studies comprising 558 cases and 907 controls were considered. In this pooled analysis, overall HPV-cuSCC association was significant with pooled OR 3.29

(CI 95%, 1.67-6.46, p < 0.001), showing that squamous cell carcinomas were more likely to carry HPV than normal-appearing skin. I^2 and Q statistics showed significant level of heterogeneity in the published studies, suggesting that individual study effect sizes varied based on different study designs, I^2 = 77.0%, Q = 43.94, p < 0.001 (Fig. 3). *INSERT F3*

Hypothesis B - HPV association with BCC *versus* normal skin

Seven studies met the eligibility criteria, resulting in a total of 370 cases and 667 controls. In our random effects pooled analysis, BCC was more likely to carry HPV than normal skin (pooled OR 2.56, CI 95%, 1.48-5.14, p = 0.008). I^2 and Q statistics showed moderate evidence of heterogeneity in the published studies, I^2 = 62.0%, Q = 15.82, p = 0.01 (Fig. 4). Removing studies one by one, we found that the exclusion of Caldeira et al[21], considerable changed the level of heterogeneity. In the absence of this article, the pooled effect was OR 1.86, CI 95%, 1.10–3.14 and I^2 = 25.0%, p = 0.25. *INSERT F4*

Hypothesis C - HPV association with cuSCC in immunosuppressed *versus* immunocompetent patients

In this pooled analysis, 444 cases and 286 controls from 12 case-controls were included. cuSCC from immunosuppressed patients was significantly more likely to carry HPV than cuSCC samples from non-immunosuppressed patients (pooled OR 3.07, CI 95%, 2.08-4.52, p < 0.001). I^2 and Q values showed no significant level of heterogeneity in the published studies, I^2 = 22.0%, Q = 14.07, p = 0.23 (Fig. 5). *INSERT F5*

Hypothesis D - HPV association with BCC in immunosuppressed *versus* immunocompetent patients

Only 3 studies (72 cases and 42 controls) were included. The random effects pooled analysis on this outcome showed a non-statistically significant difference between immunosuppression and HPV presence in BCC (pooled OR 2.38, CI 95%, 0.95-5.96, p = 0.07). I^2 and Q statistics showed no significant level of heterogeneity in the published studies, I^2 = 0%, Q = 0.06, p = 0.97 (Fig. 6). *INSERT F6*

Discussion

In our analysis studied populations varied from 23 samples (11 cases and 12 controls) to 540 samples (148 cases and 392 controls) with more than half of the studies including over 50 samples. About half of the selected case-controls were relatively recent (within the last 10 years). In general, the newer studies included broad-spectrum PCR techniques being able to detect more HPV types, specially beta-genera. Detected HPV varied from one specific HPV subtype to broad spectrum mucosal and cutaneous HPV, covering various genera.

Concerning cuSCC, these tumors were more likely to carry HPV than normal-appearing skin and immunosuppressed patients were more likely to carry HPV than immunocompetent patients. Therefore, our analysis shares similar results with the two previous identified meta-analysis on the subject.[18,19] However, Chahoud et al[19] did not included an analysis in immunosuppressed patients which represent a significant portion of the patients with cuSCC and allowed a wider range of methodologies to detect HPV, including multiplex and ELISA serologies.

Interestingly, HPV load seems to decrease during cuSCC carcinogenesis. Some studies refer a higher prevalence of HPV DNA in Actinic Keratosis (AK), a cuSCC premalignant lesion, comparing with the subsequent rates detected in cuSCC.[28] Another curious issue is that skin cells, unlike cells studied in the cervical region, do not require the expression of oncoproteins (like E6 and E7) by HPV viruses to preserve their transformed phenotype.[28] Finally, studies have failed to detect messenger RNA from beta-genus in cuSCC samples.[29] These three previous facts may suggest a temporary early role of HPV in the multistep process of oncogenesis and no need of these viruses for maintenance. We can speculate if HPV works as co-carcinogen factor with UVR. Actually, some studies have identified higher HPV rates in sun-exposed regions.[22] A local immunosuppression effect of UVR[30] or a direct trigger effect in viruses may induce apoptosis or DNA damage. This last theory was already demonstrated *in vitro* with cutaneous HPV types 5, 8, 20 and 77.[31,32]

There might also be different mechanisms driving tumor progression in HPV DNA positive versus HPV DNA negative cuSCC. Apart from the importance of studying the natural history of the carcinogenesis in HPV DNA positive cuSCC, immunological and genetic factors that are associated with HPV DNA negative tumors should also be better explored in the future.

Regarding BCC, no previous meta-analysis were found and the association with HPV was demonstrated for the first time in literature. Even without a statistically significance, BCC from immunosuppressed patients were more likely to carry HPV than tumors from immunocompetent patients.

Studies involving BCC tumours were, in general, more recent [21-23, 25, 33-38] and a lack in both quantity and quality of case-controls on BCC, particularly involving immunosuppressed patients (low number of cases and controls for hypothesis D) was found. This fact may be due to an easier logical reasoning relating cuSCC with HPV (rather than BCC), as the causality of papillomaviruses and carcinoma of the cervix in women (affecting squamous cells) is well-established. Additionally, in contrast to the immunocompetent population, cuSCC is more common in immunosuppression context than BCC.[39] New studies, covering a larger number of patients with BCC and preferentially prospective designed, would improve the quality of future meta-analysis in this matter.

The role of HPV in cutaneous carcinogenesis is far away from being completely understood. Even though our results demonstrate a strong association between HPV and NMSC, a higher prevalence of the virus in tumoral tissues does not imply causality. It is from great importance to differentiate if the HPV infection is only a simultaneously phenomenon with the occurrence of neoplasia or a major agent during the process of carcinogenesis.[40] Thus, cohort studies should be designed and performed to assess this causality, adjusting for other risk factors.

Definition of a causality link between NMSC and HPV may lead to new diagnostic and therapeutic tools. For instance, the well-established relationship between cervical carcinoma and HPV provided effective HPV vaccines and a better control of this cancer.[16] Acquired immunity through vaccination against selected HPV subtypes could potentially

be explored as an effective preventive approach, decreasing NMSC burden and further costs of care.

This meta-analysis has some limitations that should be pointed out.

Unfortunately, most of the studies did not present adjusted measures for possible confounding factors, particularly for exposure to UVR and older age, known NMSC risk factors.

Other important limitation is the great degree of heterogeneity among the literature regarding sampling methods, the laboratory technique to HPV detection and the HPV type studied. We specifically tried to minimize these points, considering only studies based on biopsy samples evaluated by experienced pathologists or PCR techniques for HPV detection rather than eyebrow pluck, skin swab or serologies.

Conclusions

This study represents, to our knowledge, the most extensive meta-analysis assessing the epidemiological association of HPV with cuSCC using PCR analysis.

Notably, this is the first meta-analysis evaluating the association between HPV and BCC. This work provides additional evidence of the eventual involvement of HPV in the development of these tumors in both immunocompetent and immunosuppressed individuals.

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Tables

Table 1

Eligibility Criteria
Article in **English, excluding abstracts, reviews and letters to the editor**
Minimum **of 10 cases and 10 controls/20 patients for determination of Odds Ratio (OR)**
Patients **without other cutaneous comorbid conditions** (Epidermodysplasia Verruciformis or other genodermatosis)
Samples including **all skin body areas except anogenital**
Biopsy samples only (rather than eyebrow pluck, skin swab or serology)
HPV detection by PCR-based methods
Outcome (at least one): - For hypothesis A **– HPV-PCR positivity in cuSCC *versus* normal skin** - For hypothesis B **– HPV-PCR positivity in BCC *versus* normal skin** - For hypothesis C **– HPV-PCR positivity in cuSCC from immunosuppressed patients *versus* cuSCC from immunocompetent patients** - For hypothesis D **– HPV-PCR positivity in BCC from immunosuppressed patients *versus* BCC from immunocompetent patients**

Table 2

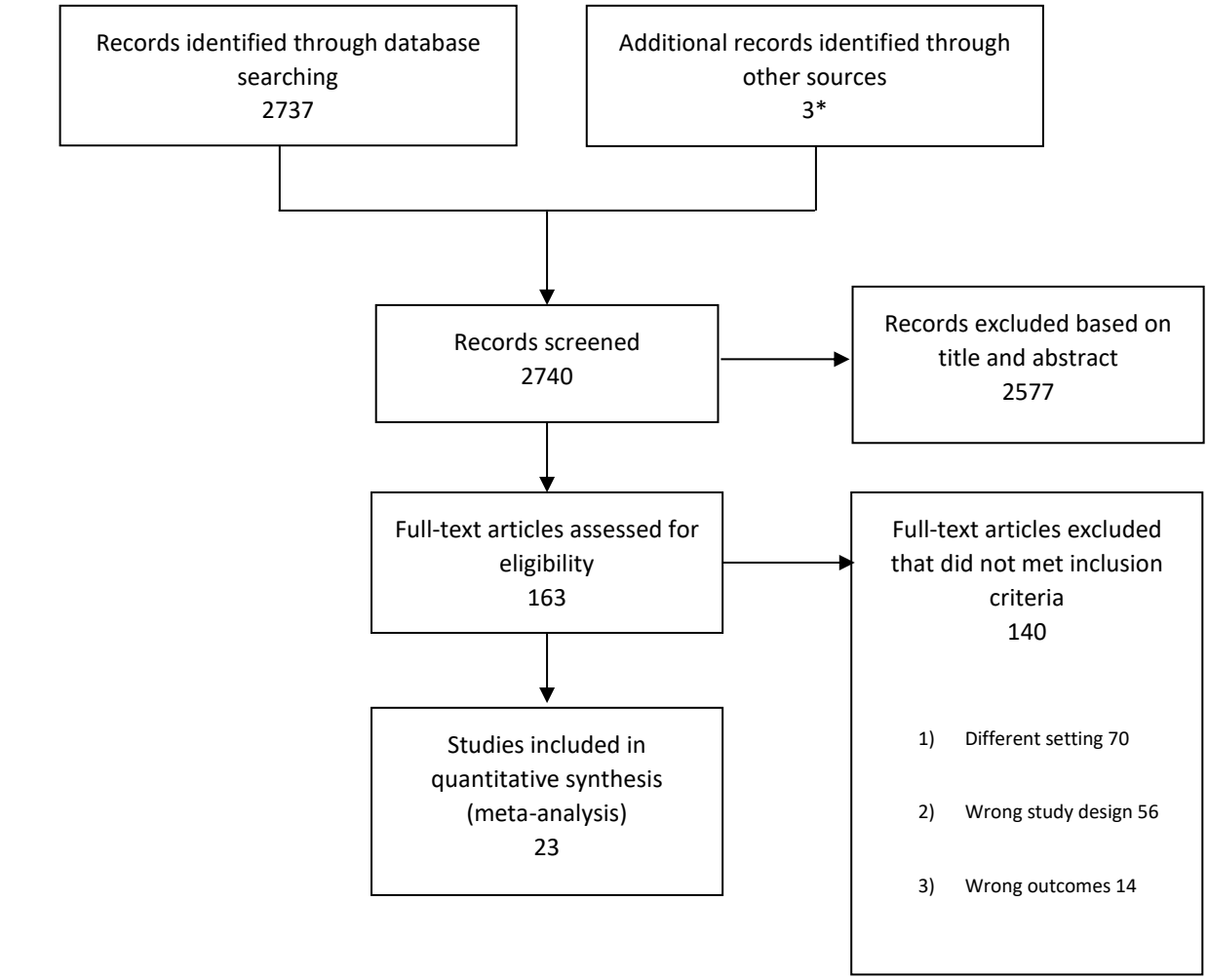
Source	Design and population (hypothesis)	HPV subtypes	Malignancies	Tumor Body Locations
Arends et al,[41] 1997	Case-control 27 cases 15 controls – C	HPV subtypes 1, 2, 5, 8, 6b, 11, 16 and 18	cuSCC	Sun-exposed and non-sun-exposed areas
Arron et al,[29] 2011	Case-control 67 cases 18 controls – A 39 cases 28 controls – C	Broad spectrum beta-HPV	cuSCC, KA type included	Sun-exposed and non-sun-exposed areas
Asgari et al,[24] 2008	Case-control 85 cases 190 controls - A	Broad spectrum HPV (including alpha, beta and gamma genera)	cuSCC	Sun-exposed and non-sun-exposed areas
Berkhout et al[33], 2000	Case-control 81 cases 31 controls – A 14 cases 31 controls – B	Broad spectrum HPV (including alpha and beta genera)	cuSCC and BCC	Sun-exposed and non-sun-exposed areas
Bernat-García et al,[25] 2014	Case-control 17 cases 17 controls – C 13 cases 13 controls – D	Broad spectrum HPV (including alpha, beta and gamma genera)	cuSCC and BCC	Sun-exposed and non-sun-exposed areas
Cairey-Remonnay et al,[42] 2002	Case-control 53 cases 51 controls - C	Broad spectrum of mucosal and cutaneous HPV	cuSCC	Sun-exposed areas
Caldeira et al,[21] 2003	Case-control 26 cases 41 controls – A 69 cases 41 controls – B	HPV subtype 38	cuSCC and BCC	ND
Escutia et al,[34] 2011	Case-control 70 cases 29 controls - B	Broad spectrum HPV (including alpha, beta and gamma genera)	BCC	Sun-exposed and non-sun-exposed areas
Forslund et al,[43] 003	Case-control 11 cases 12 controls - C	Broad spectrum of cutaneous HPV	cuSCC	Sun-exposed and non-sun-exposed areas
Forslund et al,[44] 2003, 2nd article	Case-control 60 cases 12 controls - C	Broad spectrum of cutaneous HPV	cuSCC, only KA	ND
Forslund et al,[22] 2007	Case-control 82 cases 392 controls- A 148 cases 392 controls – B	Broad spectrum HPV (including alpha, beta and gamma genera)	cuSCC and BCC	Sun-exposed and non-sun-exposed areas
Gustafsson et al,[45] 2004	Case-control 36 cases 27 controls - A	Broad anogenital HPV	cuSCC	ND
Hama et al,[20] 2006	Case-control 23 cases 17 controls- A	Broad spectrum of mucosal HPV	cuSCC	Sun-exposed and non-sun-exposed areas
Harwood et al,[35] 2000	Case-control 44 cases 22 controls – C 24 cases 11 controls – D	Broad spectrum of cutaneous, mucosal and EV HPV	cuSCC and BCC	Sun-exposed and non-sun-exposed areas
Iftner et al,[23] 2003	Case-control 72 cases 106 controls – A 18 cases 106 controls – B	Broad spectrum of mucosal and EV HPV	cuSCC and BCC	Sun-exposed and non-sun-exposed areas
Mackintosh et al,[26] 2009	Case-control 53 cases 11 controls – A 30 cases 23 controls – C	Broad spectrum beta-HPV	cuSCC, KA included in IS group	ND
Plasmeijer et al,[27] 2010	Case-control 21 cases 21 controls – A	Broad spectrum beta-HPV	cuSCC	Sun-exposed and non-sun-exposed areas
Purdie et al,[46] 2005	Case-control 84 cases 17 controls - C	Broad spectrum cutaneous HPV	cuSCC	ND
Reuschenbach et al,[36] 2011	Case-control 43 cases 44 controls – C 35 cases 18 controls – D	HPV subtypes 16, 18, 31, 33, 35,39, 45, 51, 52, 56, 58, 59, 68, 73, and 82	cuSCC, KA included and BCC	Sun-exposed and non-sun-exposed areas
Rollison et al,[37] 2008	Case-control 13 cases 15 controls - B	Broad spectrum beta-HPV	BCC	Sun-exposed and non-sun-exposed areas
Shamanin et al,[47] 1996	Case-control 20 cases 26 controls - C	Broad spectrum HPV	cuSCC	ND
Stockfleth et al,[48] 2004	Case-control 16 cases 19 controls - C	Broad spectrum mucosal, EV and classical wart-associated HPV	cuSCC	ND
Zaravinos et al,[38] 2010	Case-control 12 cases 53 controls – A 38 cases 53 controls – B	Broad spectrum HPV	cuSCC and BCC	Sun-exposed and non-sun-exposed areas

Table 3

Source	Newcastle-Ottawa Score	OR Adjustment
Arends et al,[41] 1997	**8**	--
Arron et al,[29] 2011	**8**	--
Asgari et al,[24] 2008	**7**	--
Berkhout et al,[33] 2000	**9**	--
Bernat-García et al,[25] 2014	**7**	--
Cairey-Remonnay et al,[42] 2012	**9**	--
Caldeira et al,[21] 2003	**9**	--
Escutia et al,[34] 2010	**9**	--
Forslund et al,[43] 2003	**9**	--
Forslund et al,[44] 2003, 2nd article	**9**	--
Forslund et al,[22] 2007	**9**	Adjusted for age, sex, skin type, self-reported previous sunburns, eye color and sun exposure at biopsy site
Gustafson et al,[45] 2004	**9**	--
Hama et al,[20] 2006	**9**	--
Harwood et al,[35] 2000	**9**	--
Iftner et al,[23] 2003	**5**	Adjusted for age, sex and sun exposure (sun exposed: head, face, neck, forearm, hands, and lower limb)
Mackintosh et al,[26] 2009	**5**	--
Plasmeijer et al,[27] 2010	**7**	--
Purdie et al,[46] 2005	**9**	--
Reuschenbach et al,[36] 2011	**9**	--
Rollison et al,[37] 2008	**9**	--
Shamanin et al,[47] 1996	**9**	--
Stockfleth et al,[48] 2004	**9**	--
Zaravinos et al,[38] 2010	**9**	--

Figures

Fig. 1



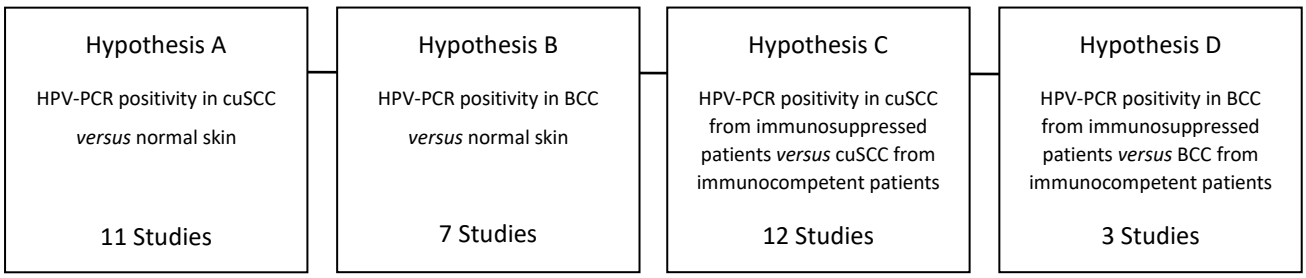


Fig. 2

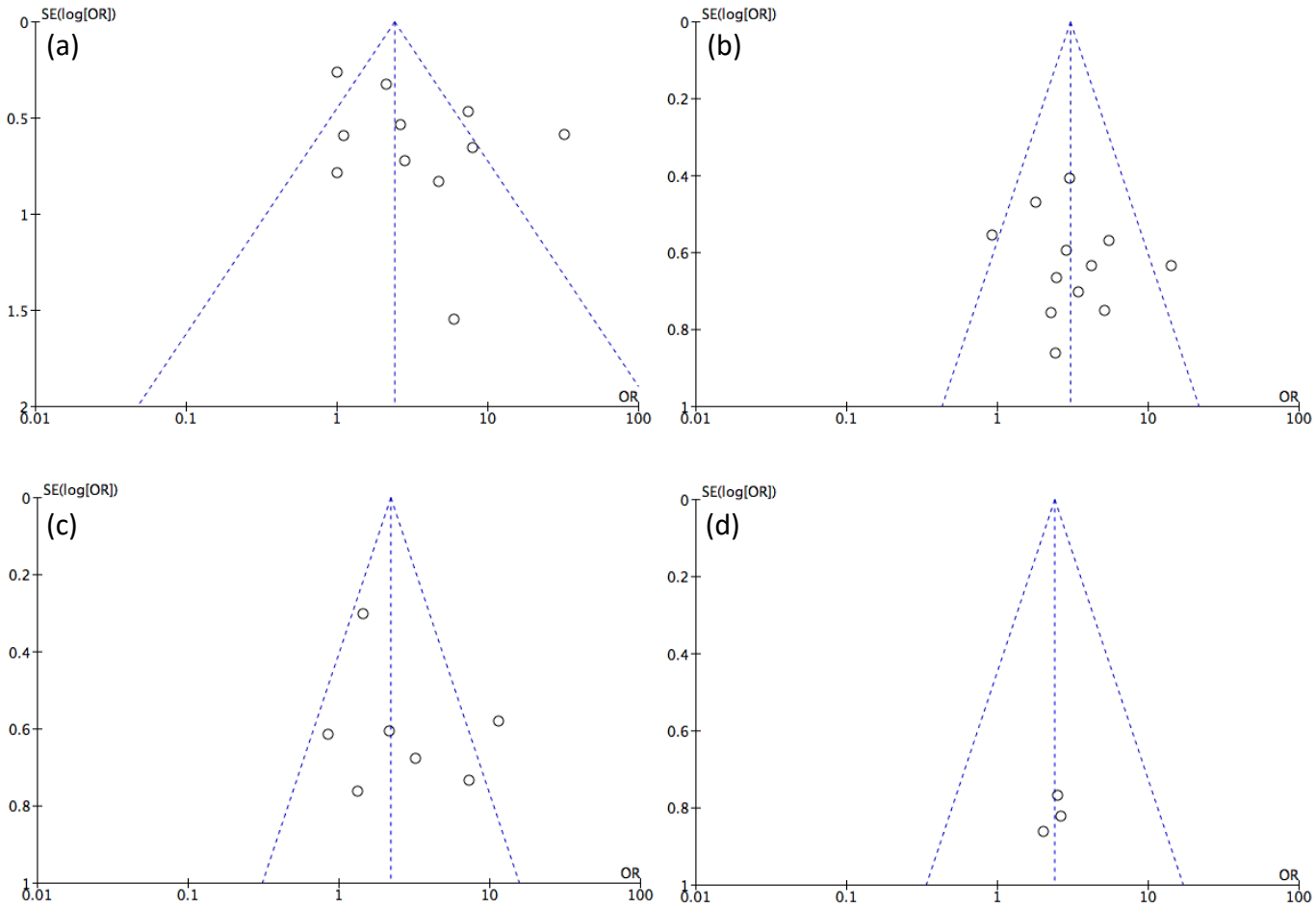

SE(log[OR])
(a)
0
0.5
1
1.5
2
0.01
0.1
1
10
100
OR
SE(log[OR])
(b)
0
0.2
0.4
0.6
0.8
1
0.01
0.1
1
10
100
OR
SE(log[OR])
(c)
0
0.2
0.4
0.6
0.8
1
0.01
0.1
1
10
100
OR
SE(log[OR])
(d)
0
0.2
0.4
0.6
0.8
1
0.01
0.1
1
10
100
OR

Fig. 3

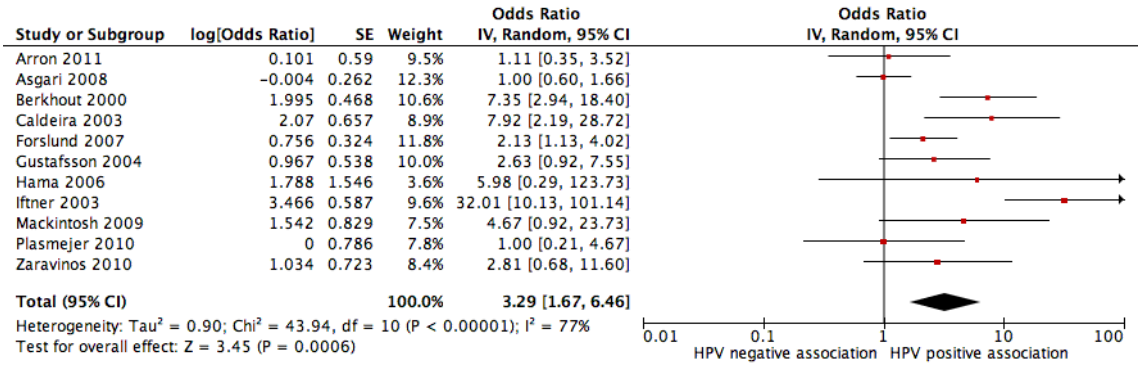

Study or Subgroup
log[Odds Ratio]
SE
Weight
Odds Ratio
IV, Random, 95% CI
Odds Ratio
IV, Random, 95% CI
Arron 2011 0.101 0.59 9.5% 1.11 [0.35, 3.52]
Asgari 2008 -0.004 0.262 12.3% 1.00 [0.60, 1.66]
Berkhout 2000 1.995 0.468 10.6% 7.35 [2.94, 18.40]
Caldeira 2003 2.07 0.657 8.9% 7.92 [2.19, 28.72]
Forslund 2007 0.756 0.324 11.8% 2.13 [1.13, 4.02]
Gustafsson 2004 0.967 0.538 10.0% 2.63 [0.92, 7.55]
Hama 2006 1.788 1.546 3.6% 5.98 [0.29, 123.73]
Iftner 2003 3.466 0.587 9.6% 32.01 [10.13, 101.14]
Mackintosh 2009 1.542 0.829 7.5% 4.67 [0.92, 23.73]
Plasmejer 2010 0 0.786 7.8% 1.00 [0.21, 4.67]
Zaravinos 2010 1.034 0.723 8.4% 2.81 [0.68, 11.60]
Total (95% CI) 100.0% 3.29 [1.67, 6.46]
Heterogeneity: Tau² = 0.90; Chi² = 43.94, df = 10 (P < 0.00001); I² = 77%
Test for overall effect: Z = 3.45 (P = 0.0006)
0.01
0.1
1
10
100
HPV negative association
HPV positive association

Fig. 4

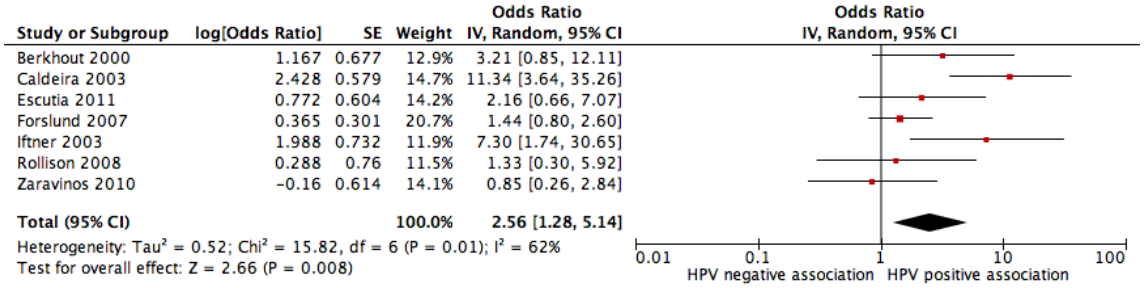

Study or Subgroup
log[Odds Ratio]
SE
Weight
Odds Ratio
IV, Random, 95% CI
Odds Ratio
IV, Random, 95% CI
Berkhout 2000 1.167 0.677 12.9% 3.21 [0.85, 12.11]
Caldeira 2003 2.428 0.579 14.7% 11.34 [3.64, 35.26]
Escutia 2011 0.772 0.604 14.2% 2.16 [0.66, 7.07]
Forslund 2007 0.365 0.301 20.7% 1.44 [0.80, 2.60]
Iftner 2003 1.988 0.732 11.9% 7.30 [1.74, 30.65]
Rollison 2008 0.288 0.76 11.5% 1.33 [0.30, 5.92]
Zaravinos 2010 -0.16 0.614 14.1% 0.85 [0.26, 2.84]
Total (95% CI) 100.0% 2.56 [1.28, 5.14]
Heterogeneity: Tau² = 0.52; Chi² = 15.82, df = 6 (P = 0.01); I² = 62%
Test for overall effect: Z = 2.66 (P = 0.008)
0.01
0.1
1
10
100
HPV negative association
HPV positive association

Fig. 5

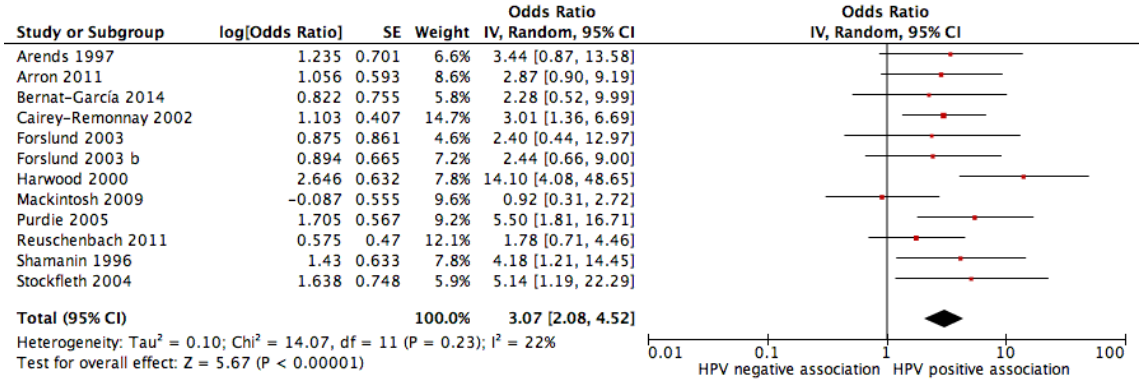
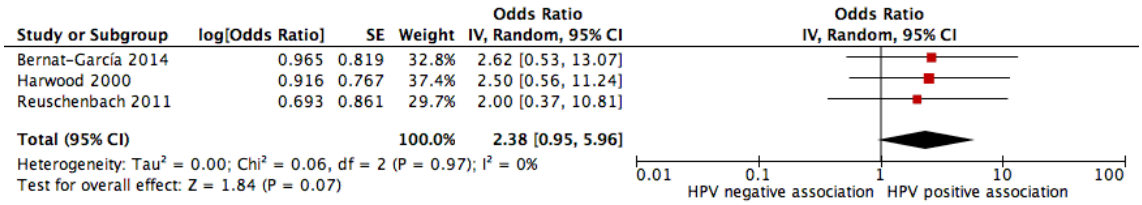

Study or Subgroup
log[Odds Ratio]
SE
Weight
Odds Ratio
IV, Random, 95% CI
Odds Ratio
IV, Random, 95% CI
Arends 1997 1.235 0.701 6.6% 3.44 [0.87, 13.58]
Arron 2011 1.056 0.593 8.6% 2.87 [0.90, 9.19]
Bernat-García 2014 0.822 0.755 5.8% 2.28 [0.52, 9.99]
Cairey-Remonnay 2002 1.103 0.407 14.7% 3.01 [1.36, 6.69]
Forslund 2003 0.875 0.861 4.6% 2.40 [0.44, 12.97]
Forslund 2003 b 0.894 0.665 7.2% 2.44 [0.66, 9.00]
Harwood 2000 2.646 0.632 7.8% 14.10 [4.08, 48.65]
Mackintosh 2009 -0.087 0.555 9.6% 0.92 [0.31, 2.72]
Purdie 2005 1.705 0.567 9.2% 5.50 [1.81, 16.71]
Reuschenbach 2011 0.575 0.47 12.1% 1.78 [0.71, 4.46]
Shamanin 1996 1.43 0.633 7.8% 4.18 [1.21, 14.45]
Stockfleth 2004 1.638 0.748 5.9% 5.14 [1.19, 22.29]
Total (95% CI) 100.0% 3.07 [2.08, 4.52]
Heterogeneity: Tau² = 0.10; Chi² = 14.07, df = 11 (P = 0.23); I² = 22%
Test for overall effect: Z = 5.67 (P < 0.00001)
0.01
0.1
1
10
100
HPV negative association
HPV positive association

Fig. 6


Study or Subgroup
log[Odds Ratio]
SE
Weight
Odds Ratio
IV, Random, 95% CI
Odds Ratio
IV, Random, 95% CI
Bernat-García 2014 0.965 0.819 32.8% 2.62 [0.53, 13.07]
Harwood 2000 0.916 0.767 37.4% 2.50 [0.56, 11.24]
Reuschenbach 2011 0.693 0.861 29.7% 2.00 [0.37, 10.81]
Total (95% CI) 100.0% 2.38 [0.95, 5.96]
Heterogeneity: Tau² = 0.00; Chi² = 0.06, df = 2 (P = 0.97); I² = 0%
Test for overall effect: Z = 1.84 (P = 0.07)
0.01
0.1
1
10
100
HPV negative association
HPV positive association

Legends

Table 1 – Eligibility criteria for systematic review.

Table 2 – Characteristics of studies meeting search eligibility criteria.

IS – Immunosuppressed; KA – Keratoacanthoma; ND – Not defined.

Table 3 - Newcastle-Ottawa scale for assessment of quality and OR adjustment of the included studies.

Fig. 1 - Flow chart of search strategy for the meta-analysis and number of studies included in final quantitative analysis by hypothesis.

* Records included in previous meta-analysis and that were not selected via research protocol.

** Some studies were included in more than one hypothesis analysis as they met criteria for both.

Fig. 2 – Funnel plots with effect measures (Odds Ratio (OR) as a function of its standard error (SE)) for the outcome of HPV positivity in studies comparing (a) cuSCC versus normal skin – Begg test: p = 0.2183; (b) BCC versus normal skin – Begg test: p = 0.8406; (c) cuSCC from immunosuppressed patients versus cuSCC from immunocompetent patients – Begg test: p = 0.7726; (d) BCC from immunosuppressed patients versus BCC from immunocompetent patients – Begg test: p = 1.0000.

Fig. 3 – Forest plot with pooled effect size and 95% confidence intervals (CI) for hypothesis A - HPV-PCR positivity in cuSCC *versus* normal skin. The squares and horizontal lines correspond to the study-specific odds ratios (ORs) and 95% CIs. The diamond shape represents the pooled OR and 95% CI of the overall population.

Fig. 4 – Forest plot with pooled effect size and 95% confidence intervals (CI) for hypothesis B - HPV-PCR positivity in BCC *versus* normal skin. The squares and horizontal lines correspond to the study-specific odds ratios (ORs) and 95% CIs. The diamond shape represents the pooled OR and 95% CI of the overall population.

Fig. 5 – Forest plot with pooled effect size and 95% confidence intervals (CI) for hypothesis C - HPV-PCR positivity in cuSCC from immunosuppressed patients *versus* cuSCC from immunocompetent patients. The squares and horizontal lines correspond to the study-specific odds ratios (ORs) and 95% CIs. The diamond shape represents the pooled OR and 95% CI of the overall population.

Fig. 6 – Forest plot with pooled effect size and 95% confidence intervals (CI) for hypothesis D - HPV-PCR positivity in BCC from immunosuppressed patients *versus* BCC from immunocompetent patients. The squares and horizontal lines correspond to the study-specific odds ratios (ORs) and 95% CIs. The diamond shape represents the pooled OR and 95% CI of the overall population.

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À minha orientadora, Professora Doutora Carmen Lisboa, por ter aceite o desafio de me orientar, pela disponibilidade inestimável e pelo constante *role model* de docência e profissionalismo ao longo dos últimos anos.

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Anexo - Normas de Publicação da Revista

Journal of the European Academy of Dermatology and Venereology (JEADV)

Editor-in-Chief: Prof. Dr. med. Dr. phil. Johannes Ring, München, Germany

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ISI Journal Citation Reports © Ranking: 2016: 9/63 (Dermatology)

Online ISSN: 1468-3083

1. AIMS & SCOPE

The *Journal of the European Academy of Dermatology and Venereology* (*JEADV*) is the official organ of the European Academy of Dermatology and Venereology (EADV).
JEADV publishes articles of general and practical interest in the field of dermatology and venereology including clinical and basic science topics, as well as research with practical implications. It does so through editorials, review and practice articles, original papers of general interest, short reports, case reports, letters to the editor, news items, features and Academy announcements.

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1. de Berker DAR, Baran R, Dawber RPR. The nail in dermatological diseases. In: *Baran and Dawber's Diseases of the Nails and their Management* (Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti A, eds), 3rd edn. Oxford: Blackwell Science Ltd, 2001; 172–92.
2. Wollina U, Hansel G. The use of topical calcineurin inhibitors in lupus erythematosus: an overview. *J Eur Acad Dermatol Venereol* 2008;**22**:1–6.
3. Graham-Brown R, Burns T. *Lecture Notes: Dermatology*. Oxford: Wiley-Blackwell, 2006.
4. British Lymphology Society. *Consensus Document on the Management of Cellulitis in Lymphoedema*. 2007. Available at: **http://www.lymphoedema.org/lsn/consensus_on_cellulitis_dec_06.pdf** (last accessed 28 November 2007).

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